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TITLE: Serum Genetic Markers as Surrogates of Prostate Cancer Progression

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CONTRACTING ORGANIZATION: John Wayne Cancer Institute
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Dave S.B. Hoon, Ph.D.

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Santa Monica, CA 90404

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Original contains colored plates: ALL DTIC reproductions will be in black and white.

14. ABSTRACT

The main purpose of the proposal is that detection of free tumor-related DNA marker(s) in serum can be used as surrogate genetic markers for monitoring ongoing events related to the pathogenesis of metastasis and provide prognostic insight into disease outcome and treatment response. The scope of the studies is to develop and validate tumor-related circulating DNA in serum of prostate cancer (PCA) patients. The goal is to validate these DNA markers. We have developed assays for circulating serum DNA in prostate cancer patients (PCA). The program has gotten on track in the past year since the approval of the Human subjects IRB. PCA patient's and normal donor serum was accrued in the past year. We have been screening for new circulating methylated and unmethylated tumor-related DNA markers. Several DNA markers were found to correlate with PCA stage significantly. Highly sensitive assays were developed to detect these DNA markers in serum. Optimal conditions to obtain high specificity and sensitivity were determined. In the coming year more PCA patients will be accrued as well as further accrual of normal age-matched donors for the study. Assays for the markers will be carried out. We plan to complete the study in the upcoming year.

15. SUBJECT TERMS
Circulating DNA, serum, prostate cancer, methylation, PCR

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Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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Body

In this grant period we began accruing PCA patients and normal donor serum for the study. This started this past year on the approval of the human subjects IRB at our institute. During the past year we have focused on accrual of blood from PCA patients and healthy male donors as outlined in the statement of work Task 1. The patients were accrued as outlined in the IRB approved protocol. Blood was processed as indicated for Task 1. The blood was semi-purified for DNA extraction and cryopreserved for later extraction or processed for DNA immediately as outlined in the proposal. The serum DNA was isolated, purified, quantified and aliquoted for future use. These procedures were optimized to obtain maximum efficiency in isolating small amounts of DNA from the serum. Various approaches were investigated to improve efficacy; these include precipitation, column extraction techniques, etc. A new beta kit (Eptitect) by Qiagen (Valencia, CA) was assessed. This kit was assessed for efficacy in DNA isolation and bisulfite modification for methylation DNA studies. Our laboratory was a beta site for the kit to be tested for tumor tissue (n = 12) from paraffin blocks and serum. We demonstrated the efficiency for paraffin-embedded tissue extraction of DNA and bisulfite conversion was better than our current precipitation extraction assay. We had achieved double or higher levels in cell-free DNA recovery. The advantage of the procedure is that it took less than 6 hrs whereas our standard approach took 2 days. Similarly, we demonstrated the assessment of extraction of DNA from patients' serum (n = 20) and bisulfite conversion was very efficient using the kit. The yield from serum was double or greater. The methylated and unmethylated DNA were assessed using specific markers by capillary array electrophoresis (CAE). The analysis demonstated that methylated DNA markers could be easily detected. We compared our standard technique and the kit side by side. The time saved from the kit was highly significant. This will allow us assessment of large number of specimens in less than one quarter of the time. This is a significant technical improvement for task 1 overall and will benefit the study overall. Most important the kit will provide better yields of serum DNA where the quantity is very low. We will continue to carry out the above procedures using the kit on all new blood specimens obtained.

In the past year we have been working on task 2 in developing and establishing serum methylated DNA markers. We have screened multiple markers that include tumor-related genes and tumor suppressor genes. In most situations individual markers were not further developed when they were not detected with any major frequency (>20%) in PCA patients serum. On all the DNA markers assessed methylated and unmethylated LINE1 repeat sequences were assessed. LINE1 are intragenic repeatitive long interdispersed sequences. The protocol involved designing and testing specific primer sequences for methylated (LINE1) and unmethylated (uLINE1). These markers were assessed in both normal donor males and PCA patients of different AJCC stages. For LINE1 we used a 297 bp (base pair) product. LINE1 in 23 normal males (>40 yrs old) and PCA stage IV patients (n=43). The analysis demonstrated there

was a significant (p=0.0048) higher copy level in PCA patients than normal donors (**Figure 1**). This demonstrated the potential significance of this DNA marker detecting PCA. A ROC (receiver operating characteristic) curve was performed to assess specificity and sensitivity. The area under the curve was (0.7211) for the comparison of normal donors and stage IV PCA patients. Next we assessed uLINE1 in serum of normal donors and PCA stage IV. In **Table 1,** we show the preliminary results on the assessment of 50 patients. Using a chi-square analysis the p value was 0.0062 with a positive predictive value (PPV) of 0.81.

Figure 1. LINE 297bp analysis

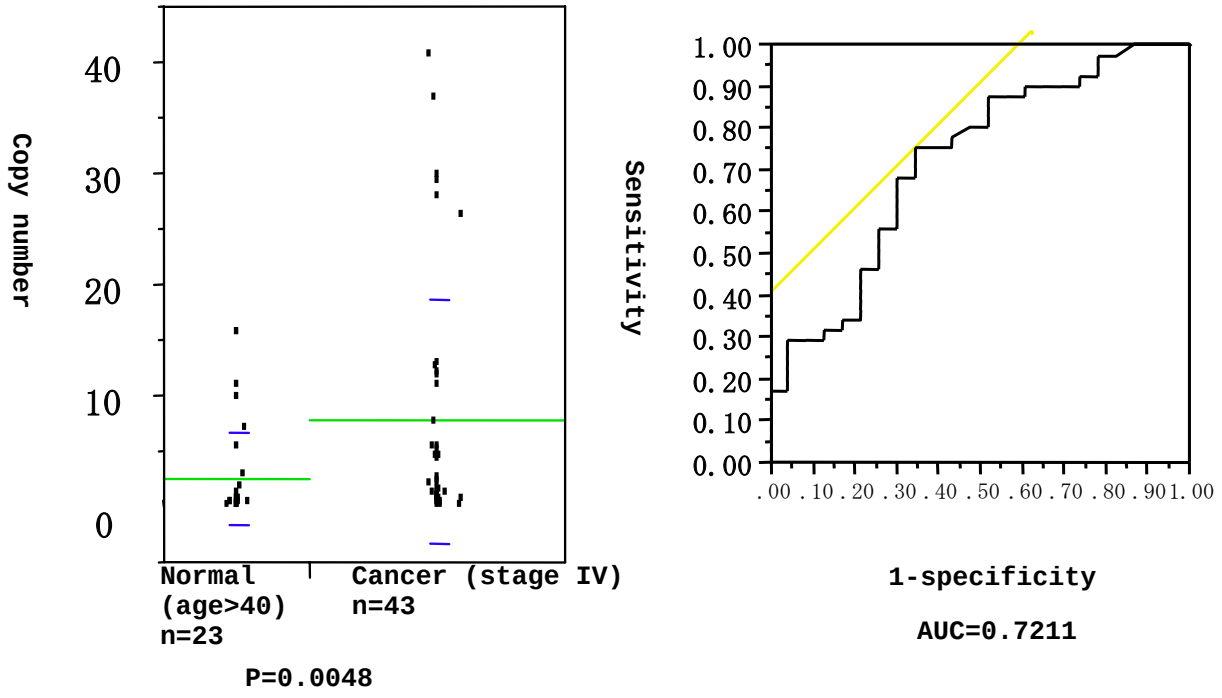

Table 1. U-LINE1 Detection in Serum

		Normal	PCA Stage IV	
U-LINE1	-	20 (87%)	14 (52%)	34
	+	3 (13%)	13 (48%)	16
		23	27	50

We assessed methylated LINE1 and unmethylated LINE1 (uLINE1) DNA in serum. Primers were designed to specific CpG island repeat sequences, PCR was performed and evaluated by CAE. Methylated LINE1 were not informative. However, we found that uLINE1 were significantly elevated in PCA patients compared to normal male donors. In **Table 2**, we show the analysis of normal serum (n=23) compared to PCA patients (stage II-IV)(n=47) serum. The comparison showed a significant (p = 0.035) difference between PCA patients than normal donors. The PPV was 0.85. These results are encouraging and will be further expanded.

Table 2. U-LINE1 Serum Detection

		Normal	PCA (All stages)	
U-LINE1	-	20 (87%)	30 (64%)	47
	+	3 (13%)	17 (36%)	23
		23	47	70

In task 4, we are collecting and processing blood from patients receiving radiation therapy. In the upcoming year we will continue accruing PCA patients and normal age matched donors for the study. Assays will be optimized for specific markers and run on the serums. New markers will be continued to be developed to improve overall sensitivity.

Key Research Accomplishments

1. PCA patients accrual for serum.
2. Normal healthy donors serum accrual for serum.
3. Blood is processed for serum and DNA is extracted.
4. DNA markers assessed for detection in serum.
5. DNA markers are optimized for specificity and sensitivity.
6. Specific DNA markers detected in PCA patients' serum.
7. Circulating DNA markers in PCA patients' serum correlated with disease stage.

Reportable Outcomes

No reportable outcomes have occurred. Currently we are writing up two manuscripts for submission.

Conclusions

The studies are back on track since the delay of the human subjects IRB approval Patients are being accrued and assays are been run on the specimens. The significance of the studies are important that we can detect circulating tumor-related DNA in serum in different forms and they appear to be related to disease progression. If successful the DNA biomarker may be of significant clinical utility in assessment of PCA patients.

References

None.

Appendices

IRB-approve letter.

Saint John's Health Center
Sisters of Charity of Leavenworth Health System

Saint John's Health Center / John Wayne Cancer Institute
Joint Institutional Review Board (IRB)

December 1, 2005

David Hoon, Ph.D.
John Wayne Cancer Institute
2200 Santa Monica Blvd
Santa Monica, CA 90404

IRB Protocol Number: HOOD-PCR-0101

Protocol Title: Serum Genetic Markers as Surrogates of Prostate Cancer: Detection of Circulating Surrogate Markers of disease in Patients Undergoing Radiation Therapy Using Polymerase Chain Reaction (PRC) and Proteomics

Principal Investigator: David Hoon, Ph.D.

Approved Co-Investigators: May Lin Tao MD, Robert Wollman, MD, Frederick R, Singer, MD, Tai Higano, MD, Steven Tucker, MD

Protocol Version Date: November 22, 2005
Consent Version Date: (Cancer Volunteer): November 22, 2005
Consent Version Date: (Cancer Free Volunteer): November 22, 2005

Re: Approval of Amendment # 6

Dear Dr. Hoon,

This is to inform you that the Saint John's Health Center/John Wayne Cancer Institute Joint IRB determined that IRB Amendment # 6 met the requirements outlined in 45 CFR 46.110 (b) (2) and 21 CFR 56.110 to receive expedited review. The Amendment included changes to study personnel.

The IRB has approved Amendment # 6 on December 1, 2005.

Amendments do not affect the regular renewal cycle. If you plan to renew this study next year, it should be renewed at least one month prior to its expiration date of **November 30, 2006.**

If you wish to continue this protocol beyond one year, you must submit the required IRB Submission Materials for Continuing Review to the IRB Office **no later than September 18, 2006**, for review in the October 4, 2006 meeting.

Any proposed change in the protocol, or consent form must be submitted to the IRB and approved prior to implementation of the change. Any death of a patient on

protocol regardless of cause must be reported in writing to the IRB within 72 hours after discovery. All serious and/or unexpected, as defined on the IRB reporting form, adverse events must be reported to the IRB in writing within 7 calendar days after discovery.

If you choose to change the status of this study during the next year, you must complete the IRB Notification of Protocol Change of Status Form, attach an explanatory cover letter and submit to the IRB Office. If all subject protocol treatment and protocol-related follow-up has ended and the study therefore is "completed" or "cancelled" in terms of this IRB, you must also complete the IRB Final Report Form

Thank you for your cooperation in our joint effort to protect the human subjects involved in our research studies.

Sincerely,

Kristin Allen, MPH, CIP
Administrator - SJHC / JWCI Joint IRB

Cc: Doug Maier, JWCI